Articles

Diabetic Nephropathy
Mechanisms of Mesangial Matrix Expansion

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Diabetic nephropathy, a major cause of morbidity and mortality in patients with diabetes mellitus, is characterized by the progressive expansion of mesangial matrix that ultimately occludes glomerular capillaries. Multiple factors in the abnormal metabolic milieu of diabetes contribute to the development of increased amounts of mesangial matrix. Glucose stimulates an increase in synthesis of most collagens and matrix glycoproteins normally expressed within the mesangium. Abnormal glycosylation of matrix proteins interferes with their degradation and turnover. Periods of hyperinsulinemia and alterations in angiotensin II induce changes in the phenotype of mesangial cells and the composition of matrix they secrete. Together, glucose, insulin, and angiotensin II conspire to produce an unrelenting increase in accumulation of mesangial matrix, with altered composition and function.

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Glomerular hypertrophy and thickening of the glomerular basement membrane with proportional enlargement of the glomerular mesangium develop in all patients with diabetes mellitus.[1,2] Yet, persons destined to have end-stage renal disease from diabetes have progressive expansion of the mesangium, which ultimately occludes the glomerular capillaries.[2] Considerable attention has recently focused on factors present in the altered metabolic milieu of diabetes that lead to mesangial matrix expansion and altered mesangial cell function. Hyperglycemia, the hallmark of diabetes mellitus, is an obvious possible contributor to many complications of diabetes, including nephropathy. Although hyperglycemia alone induces glomerular hypertrophy and thickening of basement membranes, it is insufficient to cause progressive mesangial matrix expansion.[3] Other factors (such as insulin and angiotensin II) likely interact with hyperglycemia to alter the composition of the extracellular matrix (ECM) and contribute to the development of end-stage diabetic nephropathy.

The treatment of hyperglycemia with exogenous insulin protects patients from the acute morbidity and mortality of uncontrolled diabetes and slows the rate of progression of several complications of diabetes, including nephropathy.[4,5] Such treatment has not prevented the development of nephropathy, however. Although the goal of insulin therapy is to establish normal circulating insulin levels, this is usually not achieved. Most patients experience frequent periods of hyperglycemia and hyperinsulinemia. As a result, secondary changes in circulating and locally produced growth factors commonly occur. Diabetic nephropathy develops in this complex milieu over many years. Thus, it is likely that many factors in the disordered metabolism of imperfectly treated diabetes mellitus conspire to produce unrelenting mesangial matrix expansion and, ultimately, chronic renal failure.

Changes in the Kidneys

Shortly after the onset of hyperglycemia, whole-kidney and glomerular hypertrophy develop.[6,7] Thereafter, progressive thickening of the glomerular and tubular basement membranes occurs due to the increased accumulation of matrix proteins normally present in these structures.[6,8] Later, nonenzymatic glycosylation further alters basement membrane structure and affects the barrier function of the capillary wall.[9] These changes are consistently observed in humans and animals with either type I or type II diabetes mellitus, as well as in models where galactose is administered.[3] In models of insulin resistance and in diabetic animals given exogenous insulin, the mesangial matrix progressively expands.[6,10] A similar occurrence is noted in normal animals treated with insulin. The mesangium expands by the accumulation of proteins normally present in the mesangial matrix, and new interstitial collagens and ECM proteins are expressed (Figure 1).[6,11-13] These changes steadily progress so that the amount and

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ABBREVIATIONS USED IN TEXT

ECM = extracellular matrix
IGF-I = insulin-like growth factor I
mRNA = messenger RNA
TGF-β = transforming growth factor β

composition of the mesangial matrix is greatly changed by the late stages of disease manifested by the occlusion of adjacent capillary loops.[2,6] Current research is focused on understanding those factors present in the altered metabolic milieu of diabetes that cause these qualitative and quantitative changes in mesangial matrix proteins.

Mesangium

The mesangium provides the central support for the glomerular capillaries and is composed of mesangial cells and the ECM they secrete. Normal mesangial matrix is primarily composed of collagens IV and VI, laminin, fibronectin, thrombospondin, and chondroitin sulfate proteoglycans.[6] Considerable new information has expanded our knowledge of the importance of the ECM in providing structural support for tissues and in promoting changes in cellular differentiation and cell function (reviewed by Juliano and Haskill[14] and Lin and Bissell[15]). As this field has grown, it has become apparent that collagen IV, thrombospondin, laminin, and fibronectin are each protein families with multiple genes and alternatively spliced individual gene products. The selective expression of individual family members in different extracellular matrix compartments, as well as the regulation of isoform expression within an ECM compartment, has important consequences to cell function. For example, changes in the ratios of individual matrix proteins and the expression of specific isoforms results in a mesangial matrix with completely different structural and physical properties. Moreover, an altered ECM composition engages different sets of integrin and nonintegrin receptors, which ultimately changes cell

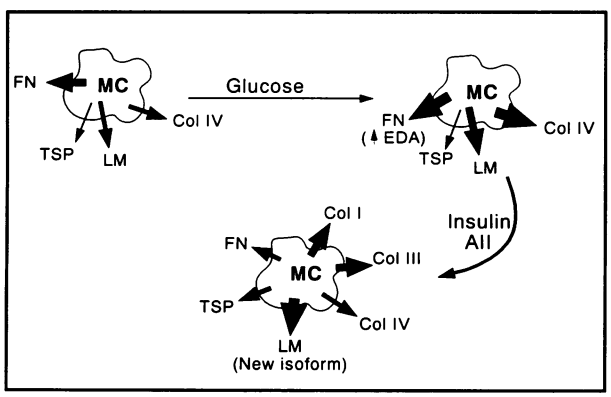

Figure 1.—The schema shows the effects of glucose, insulin, and angiotensin II (**AII**) on the amount and composition of extracellular matrix synthesized by mesangial cells (**MC**). The width of arrows indicates the relative amount synthesized. Col = collagen, EDA = extra domain A, FN = fibronectin, LM = laminin, TSP = thrombospondin

function and cellular response to cytokines and growth factors.[14] The lesion of diabetic nephropathy includes an increased accumulation of collagen IV, fibronectin, laminin, and thrombospondin in addition to the new expression of interstitial collagens I and III and novel isoforms of fibronectin and laminin.[16,17] Although additional factors undoubtedly contribute to diabetic nephropathy, the roles of glucose, insulin, and angiotensin II in promoting mesangial changes will be the focus of this review.

Regulation of Mesangial Cell Synthesis of Extracellular Matrix

Glucose

Elevated glucose levels slow the cellular proliferation of cultured mesangial cells and increase messenger RNA (mRNA) expression, protein synthesis, and protein accumulation of ECM proteins, including fibronectin, laminin, and collagens I, III, IV, and VI.[18-21] In a recent comprehensive study, glucose was shown to directly increase the rate of synthesis of all ECM proteins normally synthesized by the cell.[22] In addition, nonenzymatic glycosylation of ECM proteins was shown to slow the rate of degradation that would contribute to their accumulation in diabetes.[22] Glucose leads to an increase in diacylglycerol mass and activates protein kinase C.[23] Because diacylglycerol analogues and other activators of protein kinase C also increase ECM protein and mRNA expression by mesangial cells,[23,24] some of the direct effects of glucose on ECM synthesis appear to be through the protein kinase C pathway. In addition to the direct effects of hyperglycemia on ECM synthesis by mesangial cells, indirect effects also play a role. Hyperglycemia is associated with increased transforming growth factor-β (TGF-β) expression in mesangial cells, and TGF-β increases collagen IV and laminin synthesis.[16] These changes are partially corrected by insulin treatment.[16] These studies support a role for hyperglycemia in the increased synthesis of many ECM proteins that accumulate within the mesangium of diabetic kidneys. Improved glycemic control in patients with diabetes is therefore a necessary goal in the attempt to slow the rate of progression of diabetic renal disease.

Insulin

Studies of animals in our laboratory have shown that hyperglycemia is associated with an increased accumulation of ECM proteins normally present in the mesangium. Surprisingly, insulin treatment was found to induce a qualitative change in the collagenous composition of the ECM.[6] Because this suggested that insulin directly stimulates collagen synthesis, we examined mesangial cells in culture that were grown in the presence or absence of insulin. We found that high insulin concentrations (1 μmol per liter) stimulate mesangial cell proliferation[25,26] and cause the cells to synthesize predominantly collagens I and III.[26-28] In contrast, mesangial cells grown in the absence of insulin produce primarily collagen IV, which more closely resembles normal mesangial

matrix composition. An interesting finding was that mesangial cells that were established with insulin, but later had the insulin withdrawn, failed to synthesize the normal collagen pattern—that is, predominantly collagen IV. Thus, insulin was not only responsible for the changes in collagen expression, but the prolonged effect confirmed that the cells had undergone a phenotypic change. The finding that mesangial cells express abundant insulin receptors when propagated in media free of insulin further supports the notion that insulin and not the structurally related insulin-like growth factors (IGFs) initiated the change in collagen expression.

In addition to changes in collagen composition, diabetic nephropathy is associated with changes in the isoform of fibronectin and laminin that accumulates within the mesangium. The accumulation of the extra domain A–containing isoform of fibronectin has been shown in diabetic animals and in humans to have been influenced by glucose and insulin treatment.[16] Furthermore, we have shown that treating mesangial cells in culture with insulin induces a shift in the isoform of laminin that is synthesized.[17] Studies of laminin isoform in the mesangium in diabetic nephropathy have not been conducted. Based on the preceding data, we think that mesangial cells are responsive to physiologic concentrations of insulin and that during periods of hyperinsulinemia, insulin-mediated changes in ECM amount and composition occur. Like the results of the Diabetes Control and Complications Trial,[5] these findings support good glycemic control with frequent, low doses of insulin to avoid wide swings in both blood glucose and circulating insulin levels.

Angiotensin II

In addition to the direct effects of insulin on collagen, fibronectin, and laminin synthesis, insulin may act in concert with other factors to determine the ultimate amount and composition of ECM expressed by mesangial cells. One factor that has received considerable attention recently is angiotensin II. Studies in humans that demonstrate that the rate of progression of diabetic nephropathy can be slowed by angiotensin-converting enzyme inhibitors have prompted the evaluation of additional actions of angiotensin II. Early studies of mesangial cells in culture showed that angiotensin II–induced contractility required insulin in the medium.[29] More recently it was shown that insulin treatment of mesangial cells increases angiotensin II–receptor expression and increases the sensitivity of angiotensin II–induced stimulation of calcium channel transport.[30] Thus, insulin may augment all angiotensin II–mediated actions on mesangial cells. Angiotensin II stimulates the transcription and protein synthesis of collagen I, but not collagen IV,[31] and it increases the levels of IGF-I, platelet-derived growth factor A, basic fibroblast growth factor, and TGF-β synthesis in vascular smooth muscle cells.[32,33] These last-named stimulatory effects of angiotensin II could lead to continued alterations in the amount and type of matrix proteins that accumulate in diabetic nephropathy, because each of these growth factors has been shown to regulate mesangial cell proliferation and ECM synthesis in vitro.[34,35] For example, we have shown that IGF-I increases collagen I, III, and IV synthesis by mesangial cells,[28] but only in mesangial cells that have had long-term exposure to insulin. These in vitro effects of angiotensin II provide an important rationale for treatment trials in which angiotensin II levels are reduced by the administration of angiotensin-converting enzyme inhibitors.[36]

Summary

Progressive mesangial matrix expansion that leads to end-stage renal disease in patients with diabetic nephropathy develops slowly after many years of exposure to a complex, abnormal metabolic milieu. Many factors undoubtedly interact to produce this lesion (see Figure 1). Evidence from cell culture, animal models, and patients with diabetes mellitus shows that many factors in the diabetic milieu contribute to an increased synthesis and accumulation of ECM proteins normally present in mesangial matrix, as well as the appearance and accumulation of ECM components not normally present. The exposure of mesangial cells to insulin can induce a new expression of collagens I and III with a reduction in the rate of synthesis and the incorporation of collagen IV into the ECM. Glucose-mediated increases in fibronectin synthesis are reduced by insulin treatment, as is the expression of the extra domain A–containing isoform of fibronectin. Insulin increases the synthesis of laminin and thrombospondin and specifically induces a change in the isoform of laminin. Thus, under the influence of insulin, a mesangial matrix of entirely different composition accumulates. Periods of increased intrarenal synthesis of angiotensin II likely further stimulate the accumulation of some of these matrix proteins, specifically collagen I and laminin. Exposure to hyperglycemia augments the synthesis of all matrix components, those normally present and newly expressed ones. Together these factors lead to the steady accumulation of mesangial matrix of abnormal composition. During periods of continued abnormalities in glucose, insulin, angiotensin II, and other growth factors, a vicious cycle of increased synthesis of mesangial matrix with altered composition steadily progresses toward the ultimate occlusion of the glomerular capillary loop. Mesangial cells normally synthesize matrix-degrading enzymes that degrade type IV collagen and matrix glycoproteins, but not enzymes that degrade interstitial collagens; thus, they may preferentially accumulate. Abnormal cross-links between ECM proteins that result from nonenzymatic glycosylation may further impair the degradation of these proteins. Additional studies are needed to define the role that matrix-degrading enzymes play in the progressive expansion of mesangial matrix in diabetes mellitus.

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